

# Clinical cross-over comparison of mid-dilution hemodiafiltration using a novel dialyzer concept and post-dilution hemodiafiltration

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## Clinical cross-over comparison of mid-dilution hemodiafiltration using a novel dialyzer concept and post-dilution hemodiafiltration.

**Background.** Several studies have indicated that the improved elimination of middle molecules by convective renal replacement procedures might be associated with a better outcome in end-stage renal disease (ESRD). On-line mid-dilution hemodiafiltration (HDF) with the Nephros OLpür™ MD 190 hemodiafilter represents a novel extracorporeal renal replacement therapy concept to increase the removal of middle molecules.

**Methods.** In a prospective cross-over study in 10 ESRD patients, this technique was compared to on-line post-dilution HDF with a conventional synthetic high-flux dialyzer, operated at its technical limit, concerning small and middle molecular solute removal. Each patient was treated 3 times for  $4.0 \pm 0.4$  hours with both filters. Blood flow was 400 mL/min, substitution flow ( $Q_s$ ) during mid-dilution HDF 200 mL/min, and during post-dilution HDF 100 mL/min, and effective dialysate flow of  $700 - Q_s$  mL/min. Instantaneous clearances, reduction ratios (RR), and middle molecule mass transfer in continuously collected dialysate were determined.

**Results.** While urea and creatinine clearances were significantly lower (6.4% and 3.9%, respectively), middle molecule removal was much more efficient in mid-dilution HDF over the whole range of investigated proteins: compared to post-dilution HDF,  $\beta_2$ -microglobulin (11.8 kD) clearance ( $165.8 \pm 26.59$  vs.  $201.9 \pm 20.63$  mL/min;  $P < 0.001$ ), RR ( $80.0 \pm 5.4\%$  vs.  $82.2 \pm 5.7\%$ ;  $P < 0.001$ ), and dialysate mass transfer (53% higher;  $P < 0.001$ ) were significantly higher. For the larger middle molecules, cystatin C (13.4 kD) and retinol-binding protein (21.2 kD), mid-dilution HDF resulted in an even more superior performance, indicated by significantly higher values of all investigated parameters.

**Conclusion.** On-line mid-dilution HDF with the Nephros OLpür™ MD 190 hemodiafilter appears to be a true techno-

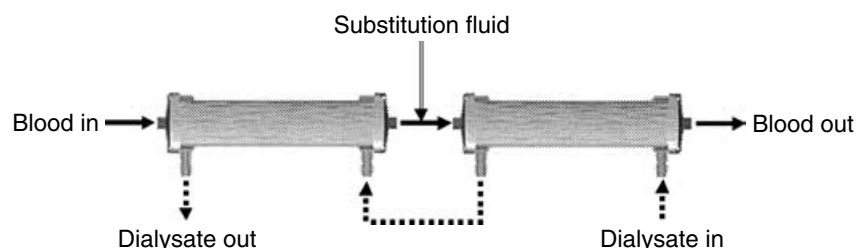
logic step ahead in terms of improved middle molecule removal. This efficient procedure gives hope to play a role in preventing or at least retarding dialysis-related long-term complications, such as  $\beta_2$ m amyloidosis, in ESRD patients, and may contribute to a more adequate dialysis therapy.

At present, about 90 different uremic toxins have been identified, and this number certainly represents only a minority of toxins that accumulate during chronic renal failure and contribute to the uremic syndrome [1]. Uremic toxins comprise a wide range of molecular weights. With low-flux hemodialysis (HD), small non-protein-bound solutes diffuse rapidly, and can pass easily through low-flux membranes while retaining middle- (500–15,000 Da) and large- (>15 kD) molecular weight molecules, also classified as low-molecular-weight (LMW) proteins. High-flux HD, using a more porous high-flux membrane, allows larger molecules to pass through the membrane. However, the quantity removed becomes internal filtration limited.

Compared to high-flux HD, hemodiafiltration (HDF) increases removal of LMW proteins such as  $\beta_2$ -microglobulin ( $\beta_2$ m) by adding an enhanced convective component to the solute mass transfer process. Post-dilution on-line HDF, representing the most efficient dialysis procedure in clinical routine, can achieve an 83% improvement in  $\beta_2$ m clearance and a 46% higher  $\beta_2$ m reduction ratio when compared with high-flux HD [2]. The efficiency of post-dilution HDF increases with the infusion rate of substitution fluid, but becomes limited by hemoconcentration and high blood viscosity as plasma water is continually ultrafiltered along the length of the dialyzer hollow fibers. In an extreme case, red cell damage, protein denaturation, and clotting of the dialyzer fibers may occur [3]. Therefore, the ultrafiltration limit is proposed to not exceed a filtration fraction of 0.5 [3].

**Key words:** hemodiafiltration, mid-dilution, hemodialyzer design, on-line substitution fluid, middle molecules.

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**Fig. 1. Schematics of mid-dilution hemodiafiltration.** Substitution fluid is reinfused between two high-flux dialyzers placed in series resulting in a first post-dilution hemodiafiltration stage followed by a predilution hemodiafiltration stage.

In predilution HDF, higher infusion rates of substitution fluid are possible, which result in better blood rheology; however, this infusion mode leads to a dilution of blood side solute concentration having the consequence of reduced clearances when compared to post-dilution HDF [4].

Even daily predilution hemofiltration [5] or daily post-dilution on-line HDF at its operational limits [6] have failed to normalize pretreatment  $\beta_2$ m plasma concentrations, evoking the demand for more efficient treatment procedures and the development of adsorptive devices, such as  $\beta_2$ m adsorbers [7]. Simultaneous pre- and post-dilutional infusion has shown to improve small solute removal in hemofiltration [8–10]. This concept was introduced as early as 1978 [11]. In HDF, mixed predilution and post-dilution on-line infusion was proposed recently [12]. However, though this technique may ensure safer operating conditions, it does not achieve substantially better removal of small- and large-size solutes relative to post-dilution HDF [13].

HD with 2 dialyzers in series enhances treatment efficiency considerably when compared to conventional single dialyzer HD [14]. This filter arrangement represents the basis for a combined post- and predilution HDF technique, named mid-dilution HDF, whereby substitution fluid is infused after the first filter and before passage of the blood through the second filter (Fig. 1). In this configuration, a post-dilution HDF stage is followed by a predilution HDF stage, the effect being a high concentration gradient in the first stage for efficient removal of small molecules, and maximal ultrafiltration of plasma water in both stages for efficient removal of larger molecules by convection. Compared with post-dilution HDF, mid-dilution HDF leads to comparable small solute clearances in vitro while achieving about 80% higher  $\beta_2$ m clearance (own unpublished data), promising more adequate dialysis therapy in terms of LMW protein removal. However, mid-dilution HDF with 2 high-flux filters in series has not become part of clinical routine due to high costs and difficult handling problems. Therefore, making mid-dilution HDF feasible for routine renal replacement therapy would be an interesting objective.

In the present study, we introduce a new concept of mid-dilution on-line HDF performed in a single cartridge, the Nephros OLpür<sup>TM</sup> MD 190 diafilter (Fig. 2). Major

study aims were to demonstrate the clinical feasibility of this device and to assess its in vivo performance in a wide molecular range, particularly removal of LMW proteins.

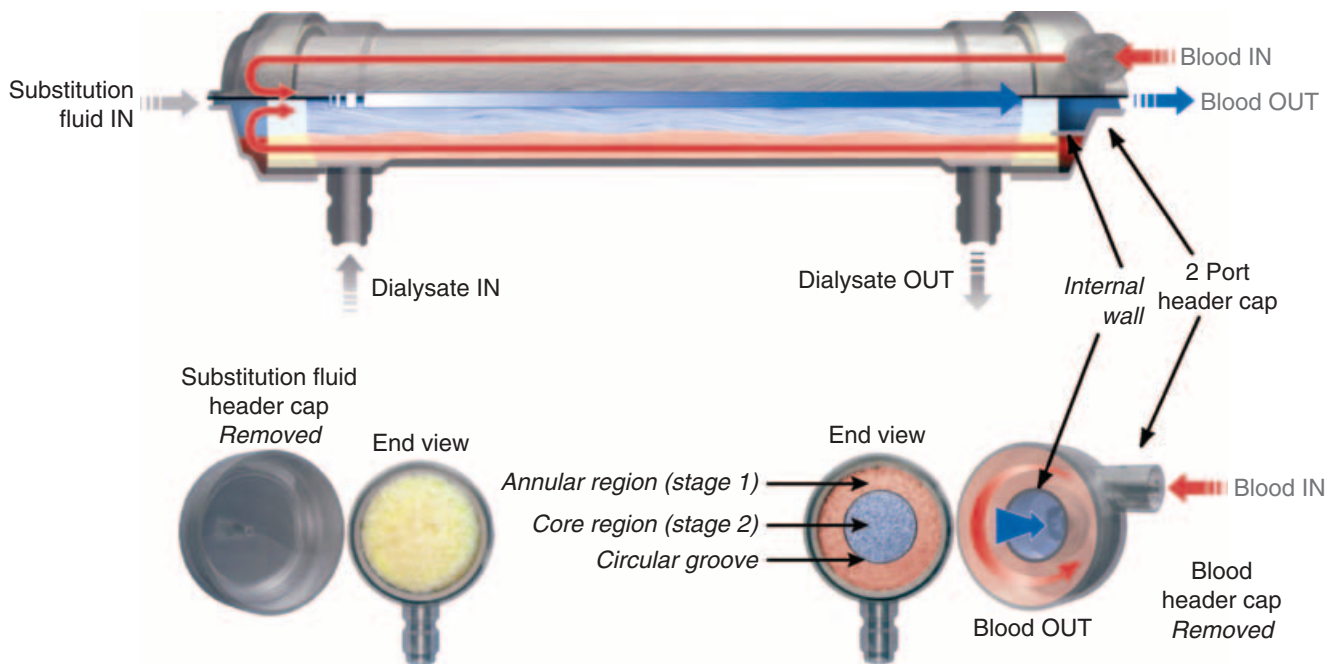
## METHODS

The study was performed according to the guidelines of Good Clinical Practice. Study approval was given by the ethics committee of Montpellier University. The study was prospective, randomized, and cross-over in nature.

Ten stable ESRD patients (mean age  $57.3 \pm 13.7$  years; 7 male, 3 female) on regular thrice weekly post-dilution HDF treatment were enrolled into the study after they had given written informed consent. Underlying renal diseases were glomerulonephritis ( $N = 6$ ), nephrosclerosis ( $N = 1$ ), hereditary dysplastic renal disease ( $N = 1$ ), Wegener's disease ( $N = 1$ ), amyloidosis ( $N = 1$ ), and unknown ( $N = 1$ ). The mean duration of dialysis treatment was  $8.3 \pm 7.77$  years (range 15 to 258 months). The mean postdialysis body weight was  $66.3 \pm 10.7$  kg, and the body mass index  $23.3 \pm 4.59$  kg/m<sup>2</sup>. All patients were anuric and had well functioning native AV fistulas for blood access. The patient's concomitant medications were continued in an unchanged manner, including heparinization for HDF under study conditions.

Each patient underwent 1 study week of 3 consecutive HDF treatments with the Nephros OLpür<sup>TM</sup> MD 190 hemodiafilter (Nephros, Inc., New York, NY, USA; high-flux polyethersulfone DIAPES<sup>®</sup> HF800, 1.9 m<sup>2</sup>; Membrana GmbH, Wuppertal, Germany) (Fig. 2) in mid-dilution mode, and 1 week of 3 consecutive HDF treatments with the control dialyzer Fresenius HF 80 S (high-flux polysulfone, 1.8 m<sup>2</sup>; Fresenius Medical Care, Bad Homburg, Germany) in post-dilution mode. The order of the 2 different treatment periods was randomly assigned to the patients. Between the 2 treatment periods, 1 week of standard HDF was carried out.

HDF sessions were performed using Gambro AK 200 ULTRA monitors equipped for on-line preparation of sterile infusion fluid (Gambro, Lund, Sweden). The HDF treatment duration was kept constant for each individual patient, and ranged between 210 and 300 minutes. One mid-dilution HDF session had to be discontinued prematurely after 175 minutes due to coagulation of the extracorporeal blood circuit. As a consequence, the



**Fig. 2. Mid-dilution hemodiafiltration in a single cartridge: the Nephros OLPur™ MD 190 hemodiafilter.** Using a special two-port header cap, blood flows through the annular region of the fiber bundle (stage 1), mixes with substitution fluid at the other end, and flows in the reverse direction through the core region of the fiber bundle (stage 2).

mean treatment duration with mid-dilution HDF was marginally shorter as compared with post-dilution HDF ( $239.8 \pm 27.7$  vs.  $243 \pm 25.3$  minutes, respectively).

Blood flow ( $Q_B$ ) and total dialysate flow ( $Q_{DT}$ ) rates were set at 400 mL/min and 700 mL/min, respectively, and were kept constant for all study sessions. The effective dialysate flow ( $Q_D$ ) entering the filter during HDF was reduced by the delivered substitution rate ( $Q_S$ ) as a consequence of on-line substitution ( $Q_D = Q_{DT} - Q_S$ ). In mid-dilution HDF,  $Q_S$  was set at 200 mL/min, while it was 100 mL/min during post-dilution HDF, resulting in a different effective dialysate flow ( $Q_D$ ) of 500 and 600 mL/min, respectively. To allow a better comparison,  $Q_S$  was chosen near the operational limit of each respective HDF mode, which was determined by previous in vitro testing for the MD 190 hemodiafilter. As a consequence, a stepwise  $Q_S$  reduction of 20 mL/min was planned when the transmembrane pressure indicated on the display of the dialysis machine during both mid-dilution and post-dilution HDF exceeded an arbitrary maximum value of 400 mm Hg. This had to be done once during the second half of the treatment in 6 of 30 mid-dilution sessions, and in 8 of 30 post-dilution sessions. The mean resulting  $Q_S$  was  $198.6 \pm 2.2$  mL/min for mid-dilution HDF, and  $98 \pm 3.5$  mL/min for post-dilution HDF.

The ultrafiltration rate ( $Q_{UF}$ ) of each session was set according to individual patient's interdialytic weight gain. Anticoagulation was performed by unchanged adoption of form and dosage of the previous routine hepariniza-

tion. Three patients received standard heparin as a bolus/continuous infusion, and 7 patients received fractionated heparin (enoxaparin; Lovenox®, Aventis Pharma, Paris, France) in a repeated bolus form.

### Quantitation of treatment efficacy

Treatment efficacy was determined by measuring reduction ratios (RR), instantaneous whole blood clearances (K), and total mass removed ( $MT_D$ ) in continuously collected dialysate. Furthermore, single pool Kt/V (spKt/V) for urea was determined [15].

All blood samples were equilibrated for 3 hours before centrifugation at 4g for 10 minutes. Plasma obtained was stored at  $-20^\circ\text{C}$  until measurement.

RR was determined for the small solute urea (60 D) and the LMW proteins  $\beta_2\text{m}$  (11.8 kD), cystatin C (CyC; 13.4 kD), and retinol-binding protein (RbP; 21.2 kD). For this purpose, plasma concentrations were measured in blood samples drawn from the arterial blood line before the start ( $C_{pre}$ ) and at the end ( $C_{post}$ ) of each treatment session after reduction of the blood flow to 50 mL/min and dialysate flow turned off for 15 seconds. RR was calculated according to equation 1 [16]. For LMW proteins,  $C_{post}$  were corrected for changes in extracellular volume [17].

$$RR = (1 - C_{post}/C_{pre}) \times 100 \quad (\text{Equation 1})$$

K was measured after 45 minutes for the small solutes urea, creatinine (113 Da), and phosphate (96 Da), and for

larger low molecular weight substances  $\beta_2m$ , CyC, and RbP. Plasma concentrations were determined in blood samples obtained from the arterial ( $C_{art}$ ) and the venous ( $C_{ven}$ ) blood line of the extracorporeal circuit. To allow optimal mixture of blood and substitution fluid, the venous samples were drawn from an additional blood line access, which was placed directly before the venous fistula needle. During sampling,  $Q_{UF}$  was maintained due to technical reasons derived from the Gambro monitors, which interrupts the substitution flow as soon as  $Q_{UF}$  is set at 0. Therefore,  $Q_{UF}$  was taken into account for calculation of  $K$  (equation 2) [18]:

$$K_{solute} = Q_B \times (C_{art} - C_{ven}/C_{art}) + (Q_{UF} \times C_{ven}/C_{art}) \quad (\text{Equation 2})$$

For reference purposes, estimates of plasma water clearances were calculated using the patient's most recent hematocrit level (Hct) and their predialysis total protein level (TP, g/L) according to equation 3. To account for the amount of solute in the red cell, solute partition coefficients (SPC) were assumed as 0.86 (urea), 0.73 (creatinine), 0.5 (phosphate), and 0 ( $\beta_2m$  and CyC).

$$K_{plasma} = K_{solute} \times (1 - 0.00107 \times TP) \times ((SPC \times Hct) + (1 - Hct)) \quad (\text{Equation 3})$$

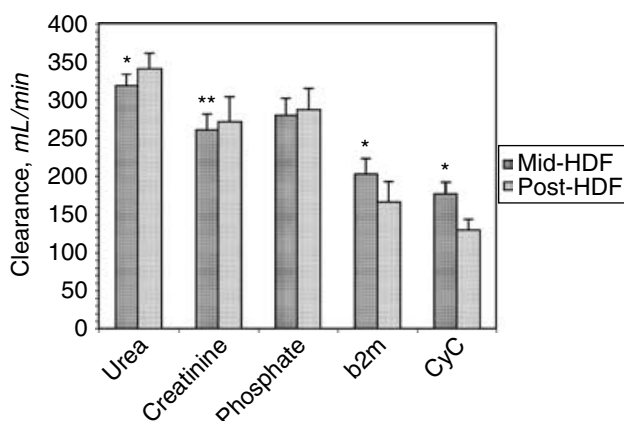
$MT_D$  was determined only during the midweek sessions. A spent dialysate fraction of 10 mL/min was collected continuously during the whole HDF duration ( $t$ ) via a T-connector inserted into the dialysate drainage line. The accuracy of the drainage volume, which consists of  $Q_D$ ,  $Q_S$ , and  $Q_{UF}$ , was verified during each session. In addition to the LMW protein concentrations ( $C_{LMW}$ ) of  $\beta_2m$ , CyC, and RbP, the albumin (67 kD) concentration was determined in an aliquot taken from the collected spent dialysate. The mass in dialysate of LMW proteins was calculated according to equation 4.

$$MT_D = C_{LMW} \times (Q_D + Q_S + Q_{UF}) \times t \quad (\text{Equation 4})$$

All LMW protein concentrations were measured by laser immunonephelometry (BN 100 Analyzer; Dade-Behring Marburg GmbH, Marburg, Germany). For the determination of RbP in dialysate, the samples were concentrated using a centrifugal filter device (Centriprep<sup>®</sup> YM-3; Millipore Corp., Bedford, MA, USA) before measurement.

## Data analysis

Descriptive analysis of the results was performed by calculating mean values  $\pm$  standard deviation (SD). Comparative statistical analyses of within-subject within-treatment changes from baseline and within-subject between-treatment differences were assessed using the two-sided paired  $t$  test. A  $P$  value of  $< 0.05$  was considered as statistically significant. Statistical evaluation was



**Fig. 3. Comparison of small solute and LMW protein clearances at 45 minutes in mid- and post-dilution HDF.** Data are mean values  $\pm$  SD ( $N = 30$ ). \* $P < 0.001$ ; \*\* $P < 0.05$ .

performed by means of the SAS software package (version 8.2; Cary, NC, USA).

## RESULTS

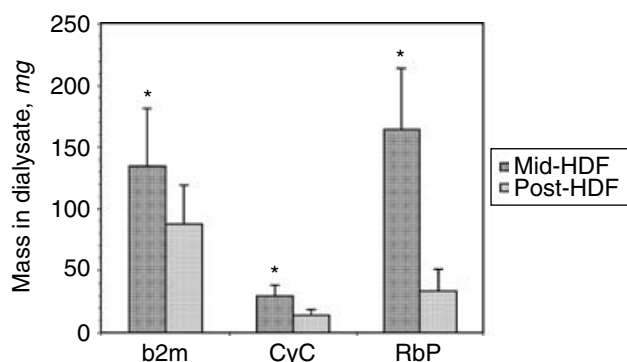
### Clinical observations

All patients completed the whole study period. During the first mid-dilution HDF treatment of 1 male patient on a repeated bolus enoxaparin anticoagulation regimen (6000 IU in total), the extracorporeal blood circuit coagulated, and the treatment had to be stopped prematurely after 175 minutes. This episode represented the only adverse event during the study. Both mid-dilution and post-dilution HDF were performed without provoking any adverse symptoms, such as headache or hypotension, in the patients.

### Treatment efficiency

Mean whole blood instantaneous clearances are reported in Figure 3. In general, post-dilution HDF led to significantly higher small solute clearances than mid-dilution HDF with the Nephros OLP<sup>®</sup> MD 190 hemodiafilter. For urea and creatinine, clearances were 6.4% and 3.9% lower in mid-dilution HDF, respectively, while no difference was found for phosphate. LMW protein clearances were substantially higher for the mid-dilution device (Fig. 3). A 21.7% clearance increase was found for  $\beta_2m$  ( $201.9 \pm 20.63$  vs.  $165.8 \pm 26.59$  mL/min). For the larger LMW protein CyC, the increase compared to post-dilution HDF amounted to even 36.9% ( $176.4 \pm 14.90$  vs.  $128.9 \pm 14.25$  mL/min). Both differences were highly significant ( $P < 0.001$ ). For RbP, negative clearance values were determined in mid- and post-dilution HDF (data not displayed).

For reference, whole blood clearances were converted to plasma water clearances as follows. For mid-dilution

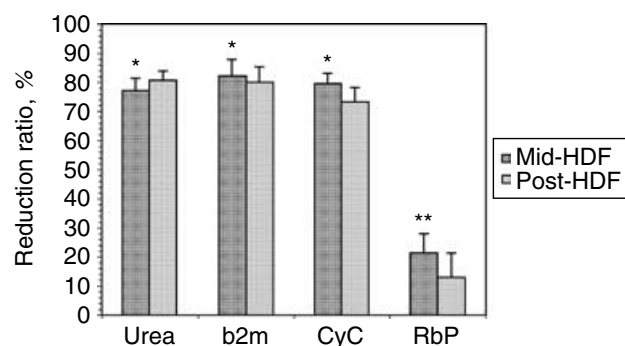


**Fig. 4.** Comparison of LMW protein mass transfer into dialysate during mid- and post-dilution HDF. Data are mean values  $\pm$  SD ( $N = 10$ ). \* $P < 0.001$ .

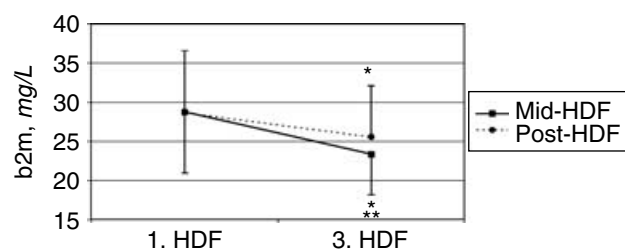
HDF with the MD 190 hemodiafilter, plasma water clearances were  $283.4 \pm 10.3$  (urea),  $220.0 \pm 14.4$  (creatinine),  $212.5 \pm 29.2$  (phosphate),  $121.4 \pm 11.9$  ( $\beta_2$ m), and  $104.9 \pm 7.5$  (CyC) compared to  $302.5 \pm 13.6$  (urea),  $228.6 \pm 18.0$  (creatinine),  $211.5 \pm 32.6$  (phosphate),  $99.5 \pm 14.7$  ( $\beta_2$ m), and  $73.2 \pm 5.0$  (CyC) mL/min for the post-dilution HDF.

Dialysate mass transfer data of LMW proteins are given in Figure 4. As expected from clearance data, substantially enhanced LMW protein removal into dialysate was measured in mid-dilution HDF as compared to post-dilution HDF. Differences in dialysate mass transfer, all of them highly significant, increased essentially with larger molecular weight. Compared to post-dilution HDF, a 53% higher mass in dialysate was determined in mid-dilution HDF for  $\beta_2$ m (11.8 kD), while it was 110% higher for CyC (13.4 kD), and 390% higher for RbP (21 kD). This trend was consistent also for albumin (67 kD). Compared to an albumin loss of  $6.32 \pm 1.63$  g with the Nephros hemodiafilter, a much lower loss of only  $0.42 \pm 0.13$  g was found in post-dilution HDF ( $P < 0.001$ ). However, corresponding to hemoconcentration due to weight loss, rising serum albumin values in the treatment course were noted in both mid- and post-dilution HDF ( $39.2 \pm 4.77$  to  $43.4 \pm 6.1$  g/L vs.  $39.6 \pm 3.16$  to  $44.8 \pm 5.4$  g/L, respectively;  $P$  not significant between mid- and post-dilution HDF).

Reduction ratios of urea and LMW proteins are displayed in Figure 5. The higher small solute clearance of post-dilution HDF was reflected by a higher urea removal of  $80.8 \pm 3.1$  vs.  $77.3 \pm 4.2\%$ . Likewise, the estimated  $\text{spKt/V}$  achieved with post-dilution HDF was  $1.99 \pm 0.21$  compared to  $1.79 \pm 0.21$  with mid-dilution HDF. A different pattern was demonstrated for LMW proteins. While  $\beta_2$ m reduction rates were generally similar in both HDF modes, mid-dilution HDF was significantly higher at  $82.2 \pm 5.7\%$  compared to  $80.0 \pm 5.4\%$  for post-dilution HDF ( $P < 0.001$ ). Plasma removal of CyC and RbP was also significantly enhanced in mid-dilution



**Fig. 5.** Comparison of urea and LMW protein plasma reduction ratios achieved by mid- and post-dilution HDF. Data are mean values  $\pm$  SD ( $N = 30$ ). \* $P < 0.001$ ; \*\* $P < 0.05$ .



**Fig. 6.** Course of mean pretreatment  $\beta_2$ m plasma concentration from first to third HDF on mid- and post-dilution HDF, respectively. Data are mean values  $\pm$  SD ( $N = 10$ ). \* $P < 0.001$  vs. first HDF; \*\* $P < 0.05$  vs. post-dilution HDF.

HDF with the OLpür<sup>TM</sup> hemodiafilter, being 8.6% higher for CyC and 67.2% higher for RbP.

Comparing mean pretreatment plasma  $\beta_2$ m before the first and the third session (i.e., after 2 treatments only), both mid- and post-dilution HDF reduced the level significantly (Fig. 6). However, mid-dilution HDF led to a significantly lower  $\beta_2$ m concentration compared to post-dilution HDF ( $28.8 \pm 7.8$  to  $23.4 \pm 5.2$  mg/L vs.  $28.7 \pm 7.9$  to  $25.6 \pm 6.5$  mg/L).

## DISCUSSION

Post-dilution HDF is regarded as the most efficient extracorporeal renal replacement therapy available for clinical routine. Efficiency of post-dilution HDF increases with the infusion rate, but it becomes limited by hemoconcentration along the dialyzer due to ultrafiltration of plasma water [3]. To overcome those limits, expensive technical modifications of the HDF procedure have been proposed, which mainly target on the safety of post-dilution HDF with only small benefits in efficiency [12, 13].

In contrast to this, on-line mid-dilution HDF with the single cartridge Nephros OLpür<sup>TM</sup> MD 190 diafilter represents a new simple concept, which substantially enhances mass removal across the spectrum of LMW proteins. Compared to post-dilution HDF, mid-dilution HDF

with this device allows the infusion of much higher substitution fluid volumes, thereby enhancing convective mass transfer without diluting solutes in blood to an extent found in predilution HDF. This last statement has been demonstrated internally through bench studies (unpublished data), whereby clearance was measured over a broad range of substitution rates and blood flow rates. In these bench studies, a positive linear correlation between clearance and substitution rate ( $Q_S$ ) was observed for urea, cytochrome C (in vitro clearance marker), and  $\beta_2m$ . During simulated testing with bovine blood ( $Q_B = 400$  mL/min,  $Q_D + Q_S = 800$  mL/min),  $\beta_2m$  clearance increased by 38 mL/min for each 100 mL/min increase in substitution rate. This net increase is similar to the difference in  $\beta_2m$  clearance observed in our clinical study, where 100 mL/min higher substitution fluid rate in mid-dilution HDF resulted in a 36.1 mL/min improvement in  $\beta_2m$  clearance when compared to post-dilution HDF.

The results of our study demonstrate that  $\beta_2m$ , as a reference middle molecule involved in dialysis-related amyloidosis, is by far better removed with mid-dilution HDF as compared to post-dilution HDF, the latter performed at its operational limit. While  $\beta_2m$  clearance was 21.7% higher,  $\beta_2m$  mass transfer into dialysate was 53% higher in mid-dilution HDF. Certainly, this finding has to be regarded with some caution because  $\beta_2m$  adsorption, which can be considerably high with synthetic dialysis membranes [19], was not taken into account. To assess adsorption, spent dialysate samples (taken at the instantaneous clearance measurement time point) were analyzed for  $\beta_2m$  for computing mass balance error. The mass balance error for both devices was, on average, a negative value suggesting some adsorption of  $\beta_2m$  on the membrane was occurring at this point during the treatment. Though the mass balance error was slightly higher for the control device (29% vs. 11%), it remains difficult to draw any significant conclusions with respect to what is happening over the entire treatment based on a single time point determination. However, enhanced  $\beta_2m$  elimination led only to slightly higher plasma removal rates, which appear to be extremely high in both mid- and post-dilution HDF when compared to literature data on HDF [2, 6]. This finding may result from limitations in the intercompartmental distribution of  $\beta_2m$ , which seems to be inadequately characterized by one- or two-pool models, and has not been investigated in patients on highly efficient HDF treatments [20, 21].

For the larger LMW proteins CyC and RbP, performance data were consistent and indicated significantly superior plasma removal rates and dialysate mass transfer in mid-dilution HDF. With increasing molecular weight, differences in treatment efficiency between mid- and post-dilution HDF rose, being 110% higher for CyC (13.4 kD) dialysate mass transfer, and a 389% larger RbP (21 kD) dialysate mass transfer than that achieved

by post-dilution HDF. This more efficient removal of LMW proteins by mid-dilution HDF opens the possibility to target even larger uremic toxins, such as complement factor D (24 kD), which is suspected to play a role in the increased rate of infectious complications in ESRD patients. Compared to high-flux hemodialysis, post-dilution on-line HDF has already shown to reduce factor D plasma levels significantly [22]. A further reduction can be expected from mid-dilution HDF. Even if the design of our short study is not appropriate to draw definitive conclusions, such expectations are supported by our data, which show a significantly lower pretreatment  $\beta_2m$  level before the third mid-dilution HDF when compared to post-dilution HDF (Fig. 6).

Additional support for the need to eliminate an enlarged spectrum of substances in order to achieve more adequate renal replacement therapy comes from 2 recent studies on super-flux dialyzers. Chronic hemodialysis with these protein-leaking devices resulted in a better control of hyperhomocysteinemia, which is associated with an increased risk for cardiovascular events in the general population, due to a presumed removal of larger solutes that influence homocysteine metabolism [23, 24]. Furthermore, super-flux hemodialysis is expected to improve renal anemia control, which might be attributed to the elimination of albumin-bound furancarboxylic acid [25]. Like super-flux dialysis, mid-dilution HDF leads to a not negligible albumin loss, which amounted to 6.3 g in our study. Whether an albumin loss of such magnitude, which is observed in peritoneal dialysis to a similar extent, can be regarded as acceptable, or even beneficial, is controversially discussed [26]. Long-term studies to define an upper limit of albumin loss of renal replacement therapies are lacking. Microinflammation and the closely associated low protein intake may be more important for the development of hypoalbuminemia than the protein loss of a particular dialysis therapy [26]. However, ESRD patients being treated with an albumin-leaking therapy form should be closely followed-up for symptoms of malnutrition, such as hypoalbuminemia and loss of body mass.

In contrast to improved LMW protein removal, urea and creatinine clearances, and subsequent treatment efficiency measures (urea reduction ratio and  $spKt/V$ ) were significantly superior in post-dilution HDF during our study. This finding was not surprising, although our own in vitro experiments revealed similar small solute clearances for both mid- and post-dilution HDF (unpublished data). The explanation may come from differences between in vitro and in vivo treatment conditions. The clinical study was performed with Gambro AK 200 ULTRA on-line HDF monitors, which is limited to a maximum dialysate flow of 700 mL/min. Compared to the clinical study, our in vitro experiments were performed using identical blood and substitution fluid flows, but the

effective dialysate flow then was 100 mL/min higher, being 600 and 700 mL/min in mid- and post-dilution HDF, respectively, as the total dialysate flow was set at 800 mL/min. The influence of the dialysate flow on small solute clearance is not linearly related. Despite the same absolute difference, a dialysate flow reduction from 600 to 500 mL/min leads to a higher decrease of small solute clearance than does a reduction from 700 to 600 mL/min [27]. Furthermore, the design of the Nephros hemodiafilter is such that a cocurrent dialysate flow exists as part of the second "predilution" stage (Fig. 2). The loss in device efficiency associated with both cocurrent dialysate flow and predilution of blood entering the second stage (which reduces the blood/dialysate concentration gradient that drives small solute diffusive clearance) is likely more affected and worsened at reduced dialysate flow rates when compared to standard HD. To achieve comparable small solute clearances in mid- and post-dilution HDF, it is therefore recommended to set dialysate flows to values of 800 mL/min, if possible. However, the dialysis dose achieved by mid-dilution HDF in our study far exceeded current dose recommendations [28].

In contrast to urea and creatinine clearances, and despite being a small solute, phosphate clearances were almost identical in both mid- and post-dilution HDF. It is a well-established fact that, due to a hydration shell surrounding the phosphate molecule, phosphate rather behaves like a middle molecular, than a small molecular, substance. Therefore, phosphate is particularly well removed by convective dialysis procedures [29].

The first clinical application of mid-dilution HDF with the OLP<sub>ur</sub><sup>TM</sup> MD 190 hemodiafilter in 10 ESRD patients over a short period of 1 week evidenced that this treatment procedure represents a feasible, easily performed extracorporeal renal replacement therapy. Compared to post-dilution HDF, it was equally well tolerated without provoking side effects in the patients. However, a single mid-dilution HDF treatment had to be stopped prematurely due to a coagulated extracorporeal blood circuit in a patient on repeated bolus enoxaparin, which was adopted in an unchanged manner from the patient's standard HDF protocol. It has to be assumed that the unique design of the Nephros hemodiafilter, with its longer, compared to a standard dialyzer, and turning blood pathway (Fig. 2), may lead to a considerably higher activation of coagulation. In fact, compared to a standard DIAPES<sup>®</sup> HF800 dialyzer, which has proven very low activation of coagulation [30], in vitro assessment in a 1-hour sham dialysis experiment has demonstrated a higher generation of thrombin-antithrombin-III complexes (15.6 vs. 86.4 ng/mL; *N* = 3), a standard biocompatibility parameter to detect the activation of the coagulation cascade. On the other hand, as presented above, mid-dilution HDF is a more efficient convective therapy in terms of LMW protein removal than post-dilution HDF. Given the fact

that all treatments were performed according to the routine procedures of the study center, fractionated heparin was initially injected by a prefabricated syringe via the arterial bloodline port when the extracorporeal circuit was filled with blood. At that moment, anticoagulation is incomplete and unbound middle molecular enoxaparin (3800 Da) could have been removed by the highly efficient mid-dilution HDF therapy. In this context, a recent study, which compared on-line predilution hemofiltration and HDF to high-flux HD, has shown that a higher convective mass transfer caused by a greater ultrafiltration volume is associated with an increased procoagulatory activity in the extracorporeal circuit [31]. However, further study treatments were uneventful without changing heparin dosages after fractionated heparin was given in all patients before connection to the extracorporeal bloodlines. This was to guarantee systemic anticoagulation prior to starting the therapy.

## CONCLUSION

Even if the HEMO study has failed to demonstrate a clear advantage of high-flux dialysis over conventional low-flux dialysis [28], convective therapies with enhanced LMW protein removal seem to improve outcome of ESRD patients [32]. On-line mid-dilution HDF with the single cartridge Nephros OLP<sub>ur</sub><sup>TM</sup> MD 190 hemodiafilter appears to be a true technologic step ahead in terms of improved LMW protein removal. This efficient device gives hope to play a role in preventing, or at least retarding, dialysis-related long-term complications, such as  $\beta_2$ m amyloidosis, in ESRD patients. However, contribution to a more adequate dialysis therapy with proven clinical benefit must be demonstrated with long-term studies.

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